SUMMARY OF SAFETY AND EFFECTIVENESS DATA

I. GENERAL INFORMATION

Device Generic Name: filler, recombinant human bone morphogenetic protein, collagen scaffold with metal prosthesis, osteoinduction

Device Trade Name: InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device

Applicant's Name and Address: Medtronic Sofamor Danek, Inc. USA
1800 Pyramid Place
Memphis, TN 38132

Premarket Approval Application (PMA) Number: P000058

Date of Panel Recommendation: January 10, 2002

Date of Notice of Approval of Application: July 2, 2002

II. INDICATIONS FOR USE

The InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device is indicated for spinal fusion procedures in skeletally mature patients with degenerative disc disease (DDD) at one level from L_4-S_1. DDD is defined as discogenic back pain with degeneration of the disc confirmed by patient history and radiographic studies. These DDD patients may also have up to Grade I spondylolisthesis at the involved level. Patients receiving the InFUSE™ Bone Graft/ LT-CAGE™ Lumbar Tapered Fusion Device should have had at least six months of nonoperative treatment prior to treatment with the InFUSE™ Bone Graft/LT-CAGE™ device. The InFUSE™ Bone Graft/ LT-CAGE™ Lumbar Tapered Fusion Device is to be implanted via an anterior open or an anterior laparoscopic approach.

III. CONTRAINDICATIONS

- The InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device is contraindicated for patients with a known hypersensitivity to recombinant human Bone Morphogenetic Protein-2, bovine Type I collagen or to other components of the formulation.

- The InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device should not be used in the vicinity of a resected or extant tumor.

- InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device should not be used in patients who are skeletally immature (<18 years of age or no radiographic evidence of epiphyseal closure).

- The InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device should not be used in pregnant women. The potential effects of rhBMP-2 on the human fetus have not been evaluated.

- The InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device should not be implanted in patients with an active infection at the operative site or with an allergy to titanium or titanium alloy.

IV. WARNINGS AND PRECAUTIONS

WARNINGS:

- Women of childbearing potential should be advised that antibody formation to rhBMP-2 or its influence on fetal development have not been assessed. In the clinical trial supporting the safety and effectiveness of the InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device, 2/277 (0.7%) patients treated with InFUSE™ Bone Graft component and 1/127 (0.8%) patients treated with autograft bone developed antibodies to rhBMP-2. The effect of maternal antibodies to rhBMP-2, as might be present for several months following device implantation, on the unborn fetus is unknown. Additionally, it is unknown whether fetal expression of BMP-2 could re-expose mothers who were previously antibody positive, thereby eliciting a more powerful immune response to BMP-2 with adverse consequences for the fetus. Studies in genetically altered mice indicate that BMP-2 is critical to fetal development and that lack of BMP-2 activity, as might be induced by antibody formation, may cause neonatal death or birth defects.

- The safety and effectiveness of the InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device in nursing mothers has not been established. It is not known if BMP-2 is excreted in human milk.

- Women of childbearing potential should be advised to not become pregnant for one year following treatment with the InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device.

- The safety and effectiveness of the InFUSE Bone Graft component with other spinal implants, implanted at locations other than the lower lumbar spine, or used in surgical techniques other than anterior open or anterior laparoscopic approaches have not been established. When degenerative disc disease was treated by a posterior lumber interbody fusion procedure with cylindrical threaded cages, posterior bone formation was observed in some instances.

- The implantation of the InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device using an anterior laparoscopic surgical approach is associated with a higher incidence of retrograde ejaculation when compared to implantation using the an anterior open surgical approach.

PRECAUTIONS:

General

- The safety and effectiveness of repeat applications of the InFUSE™ Bone Graft component has not been established.

- The InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device should only be used by surgeons who are experienced in spinal fusion procedures and have undergone adequate training with this device, for anterior laparoscopic and/or anterior open procedures.

- Two LT-CAGE™ Lumbar Tapered Fusion Device components should be implanted side by side at the surgical level whenever possible.

- The LT-CAGE™ Lumbar Tapered Fusion Device components and instruments must be sterilized prior to use according to the sterilization instructions provided in the package insert for that component, unless supplied sterile and clearly labeled as such.

- The InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device is intended for single use only. Discard unused product and use a new device for subsequent applications.

- Prior to use, inspect the packaging, vials and stoppers for visible damage. If damage is visible, do not use the product. Retain the packaging and vials and contact a Medtronic Sofamor Danek representative.

- Do not use after the printed expiration date on the label.

Hepatic and Renal Impairment

- The safety and effectiveness of the InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device in patients with hepatic or renal impairment has not been established. Pharmacokinetic studies of rhBMP-2 indicate that the renal and hepatic systems are involved with its clearance.

Geriatrics

- Clinical studies of the InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device did not include sufficient numbers of patients 65 years and older to determine whether they respond differently from younger subjects.

Bone formation

- The safety and effectiveness of the InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device has not been demonstrated in patients with metabolic bone diseases.

- While not specifically observed in the clinical study, the potential for ectopic, heterotopic or undesirable exuberant bone formation exists.

Antibody Formation/Allergic Reactions

- The safety and effectiveness of the InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device has not been demonstrated in patients with autoimmune disease.

- The safety and effectiveness of the InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device has not been demonstrated in patients with immunosuppressive disease or suppressed immune systems resulting from radiation therapy, chemotherapy, steroid therapy or other treatments.

Immunogenicity

- As with all therapeutic proteins, there is a potential for immune responses to be generated to the InFUSE™ Bone Graft component. The immune response to the InFUSE™ Bone Graft components was evaluated in 349 investigational patients and 183 control patients receiving lumbar interbody fusions.

    - *Anti-rhBMP-2 antibodies:* 2/349 (0.6%) patients receiving the InFUSE™ Bone Graft component developed antibodies vs. 1/183 (0.5%) in the control group.

    - *Anti-bovine Type I collagen antibodies:* 18.1% of patients receiving the InFUSE™ Bone Graft component developed antibodies to bovine Type I collagen vs. 14.2% of control patients. No patients in either group developed anti-human Type I collagen antibodies.

    - The presence of antibodies to rhBMP-2 was not associated with immune mediated adverse events such as allergic reactions. The neutralizing capacity of antibodies to rhBMP-2 is not known.

- The incidence of antibody detection is highly dependent on the sensitivity and specificity of the assay. Additionally, the incidence of antibody detection may be influenced by several factors including sample handling, concomitant medications and underlying disease. For these reasons, comparison of the incidence of antibodies to the InFUSE™ Bone Graft component with the incidence of antibodies to other products may be misleading.

V. DEVICE DESCRIPTION

The InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device consists of two components containing three parts– a tapered metallic spinal fusion cage, a recombinant human bone morphogenetic protein and a carrier/scaffold for the bone morphogenetic protein and resulting bone. The InFUSE™ Bone Graft component is inserted into the LT-CAGE™ Lumbar Tapered Fusion Device component to form the complete InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device. **These components must be used as a system. The InFUSE™ Bone Graft component must not be used without the LT-CAGE™ Lumbar Tapered Fusion Device component.**

LT-CAGE™ Lumbar Tapered Fusion Device component

The LT-CAGE™ device consists of a hollow, perforated, machined cylinder with opposing flat sides. The cage has a tapered design with an angle of 8.8° and is available in diameters ranging from 14mm to 18mm at the narrow end of the taper, 17mm to 22 mm at the wide end of the taper and in lengths ranging from 20mm to 26mm. There are two holes on each of the two flat sides. On each of the two rounded aspects, there is a single rounded slot. The implants have a helical screw thread on the outer surface. One end of the device is closed. The other end is open to be filled with the InFUSE™ Bone Graft component.

The LT-CAGE™ implants are made from implant grade titanium alloy (Ti-6Al-4V) described by such standards as ASTM F136 or its ISO equivalent.

The LT-CAGE™ Lumbar Tapered Fusion Device component is sold separately from the InFUSE™ Bone Graft component, however, these two components must be used together. The package labeling for the LT-CAGE™ Lumbar Tapered Fusion Device contains complete product information for this component.

InFUSE™ Bone Graft component

InFUSE™ Bone Graft consists of recombinant human Bone Morphogenetic Protein-2 (rhBMP-2, known as dibotermin alfa) placed on an absorbable collagen sponge (ACS). The InFUSE™ Bone Graft component induces new bone tissue at the site of implantation. Based on data from non-clinical studies, the bone formation process develops from the outside of the implant towards the center until the entire InFUSE™ Bone Graft component is replaced by trabecular bone.

rhBMP-2 is the active agent in the InFUSE™ Bone Graft component. rhBMP-2 is a disulfide-linked dimeric protein molecule with two major subunit species of 114 and 131 amino acids. Each subunit is glycosylated at one site with high-mannose-type glycans. rhBMP-2 is produced by a genetically engineered Chinese hamster ovary cell line.

rhBMP-2 and excipients are lyophilized. Upon reconstitution, each milliliter of rhBMP-2 solution contains: 1.5 mg of rhBMP-2; 5.0 mg sucrose, NF; 25 mg glycine, USP; 3.7 mg L-glutamic acid, FCC; 0.1 mg sodium chloride, USP; 0.1 mg polysorbate 80, NF; and 1.0 mL of sterile water. The reconstituted rhBMP-2 solution has a pH of 4.5, and is clear, colorless and essentially free from plainly visible particulate matter.

The ACS is a soft, white, pliable, absorbent implantable matrix for rhBMP-2. ACS is made from bovine Type I collagen obtained from deep flexor (Achilles) tendon. The ACS acts as a carrier for the rhBMP-2 and acts as a scaffold for new bone formation.

Three sizes of the InFUSE™ Bone Graft component are available based on the internal volume of the LT-CAGE™ Lumbar Tapered Fusion Device component that is selected. The table below lists the appropriate InFUSE™ Bone Graft kit for the corresponding LT-CAGE™ Lumbar Tapered Fusion Device component size:

InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device Combinations				
LT-CAGE™ Lumbar Tapered Fusion Device		**Appropriate InFUSE™ Bone Graft Kit**		
Part #	Size (lead diameter, mm x length, mm)	Part #	Kit name (size in cc)	Reconstituted rhBMP-2/ACS graft volume
8941420	14x20	7510200	Small (2.8)	2.8ml
8941423	14x23	7510200	Small (2.8)	2.8ml
8941620	16x20	7510200	Small (2.8)	2.8ml
8941623	16x23	7510400	Medium (5.6)	5.6ml
8941626	16x26	7510400	Medium (5.6)	5.6ml
8941823	18x23	7510400	Medium (5.6)	5.6ml
8941826	18x26	7510600	Large Pre-Cut (8.0)	8.0ml
8941826	18x26	7510800	Large II (8.0)	8.0ml

Each kit contains all the components necessary to prepare the InFUSE™ Bone Graft component: the rhBMP-2 which must be reconstituted, sterile water, absorbable collagen sponges, syringes with needles, this package insert and instructions for preparation. The number of each item may vary depending on the size of the kit.

The rhBMP-2 is provided as a lyophilized powder in vials delivering either 4.2 mg or 12 mg of protein. After appropriate reconstitution, both configurations result in the same formulation and concentration (1.5 mg/mL) of rhBMP-2. The solution is then applied to the provided absorbable collagen sponge(s). The InFUSE™ Bone Graft component is prepared at the time of surgery and allowed a prescribed amount of time (no less than 15 minutes) before placement inside of the LT-CAGE™ Lumbar Tapered Fusion Device components. The Instructions for Preparation contain complete details on preparation of the InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device.

No other warranties, express or implied, are made. Implied warranties of merchantability and fitness for a particular purpose or use are specifically excluded.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

Non-surgical alternatives to performing interbody fusion with the InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device include, but are not limited to,

watchful waiting with no intervention, physical therapy, medications, external bracing, chiropractic care, spinal injections, bed rest and exercising.

Surgical alternatives include posterior lumbar interbody fusion (PLIF) procedures with or without instrumentation, anterior lumbar interbody fusion (ALIF) procedures with or without instrumentation, combined anterior and posterolateral (360°) fusion procedures, fusions using anterior/anterolateral spinal systems (e.g., plate and screw systems) or fusions using posterior spinal systems (e.g., pedicle screw/rod and hook/rod systems). In each case the fusions would involve the use of autograft and/or allograft bone.

VII. MARKETING HISTORY

The InFUSE™ Bone Graft components, manufactured by Genetics Institute, Inc., has only been used in IDE studies in the United States.

The LT-CAGE™ Lumbar Tapered Fusion Device component, manufactured by Medtronic Sofamor Danek, has been distributed in the United States since September, 1999 and it has not been withdrawn from marketing for any reason. Since 1995, this device component has been distributed outside of the United States and it has not been withdrawn from marketing for any reason.

The InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device has not been marketed for the combined use described in the PMA in the United States or any foreign country.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

The InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device was implanted in 288 investigational patients and compared to 139 control patients who received an LT-CAGE™ Lumbar Tapered Fusion Device filled with iliac crest autograft. The investigational patients were implanted with the device via either an open anterior surgical approach or a laparoscopic anterior surgical approach. The control patients were implanted only via the open anterior surgical approach.

Adverse event rates presented are based on the number of patients having at least one occurrence for a particular adverse event divided by the total number of patients in that treatment group.

ADVERSE EVENTS

(InFUSE™ Bone Graft/LT-Cage™ Device data combined from all experience with the device)

	Surgery		Postoperative (1 day - <4 Weeks)		6 Weeks (³ 4 Wks - <9 Weeks		3 Months (³ 9 Wks - <5 Months)		6 Months (³ 5 Mos - <9 Months)		12 Months (³ 9 Mos - <19 Months)		24 Months (³ 19 Mos - <30 Months)		# of Patients Reporting & Total adverse events	
Complication	Inves.	Control	Inves.	Control	Inves.	Control	Inves.	Control	Inves.	Control	Inves.	Control	Inves.	Control	Investigational # (% of 288) total events	Control #(% of 139) total events
Anatomical/Technical Difficulty	10	3	0	0	0	0	0	0	0	0	0	0	0	0	10 (3.5) 10	3 (2.2) 3
Back and/or Leg Pain	0	0	1	4	11	5	10	5	14	4	20	7	6	8	65 (22.6) 72	30 (21.6) 33
Cancer	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1 (0.3) 1	1 (0.7) 1
Cardio/Vascular	2	0	4	5	6	2	1	3	2	1	3	2	0	1	15 (5.2) 18	12 (8.6) 14
Death	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0 (0.0) 0	1 (0.7) 1
Dural Injury	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0 (0.0) 0	1 (0.7) 1
Gastrointestinal	1	0	38	22	2	0	5	1	7	1	9	3	4	5	53 (18.4) 67	27 (19.4) 32
Graft Site Related	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0 (0.0) 0	8 (5.8) 8
Implant Displacement/ Loosening	0	0	1	1	3	0	1	0	0	0	0	0	0	0	5 (1.7) 5	1 (0.7) 1
Infection	0	0	19	9	8	4	4	1	5	1	3	0	0	2	35 (12.2) 39	16 (11.5) 17
Malpositioned Implant	5	0	0	0	0	0	0	0	0	0	0	0	0	0	5 (1.7) 5	0 (0.0) 0
Neurological	0	0	7	5	7	3	5	2	5	2	10	3	5	7	36 (12.5) 39	21 (15.1) 22
Non-Union	0	0	0	0	0	0	1	0	1	3	2	0	1	1	5 (1.7) 5	4 (2.9) 4
Other	6	6	17	11	7	2	3	4	8	4	14	8	9	8	50 (17.4) 64	37 (26.6) 43
Other Pain	0	0	1	1	2	0	4	2	5	1	7	6	6	3	21 (7.3) 25	12 (8.6) 13
Respiratory	0	0	3	2	1	0	0	0	1	0	0	1	0	1	5 (1.7) 5	4 (2.9) 4
Retrograde Ejaculation	0	0	4	1	5	0	1	0	0	0	2	0	0	0	11 (7.9)[1] 12	1 (1.4)[2] 1
Spinal Event	0	0	1	2	0	0	6	2	8	3	8	8	4	2	24 (8.3) 27	16 (11.5) 17
Subsidence	0	0	3	2	2	0	1	0	1	0	0	0	0	0	7 (2.4) 7	2 (1.4) 2
Trauma	0	0	4	4	5	3	11	6	14	5	27	9	11	7	60 (20.8) 72	29 (20.9) 34
Urogenital	1	0	20	5	2	0	2	2	6	1	2	1	4	2	33 (11.5) 37	10 (7.2) 11
Vascular Intra-Op	15	5	0	0	0	0	0	0	0	0	0	0	0	0	14 (4.9) 15	5 (3.6) 5
Vertebral Fracture	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1 (0.3) 1	0 (0.0) 0
Any Adverse Event															213 (74.0)	112 (80.6)

The reported rates of several adverse events were high, but similar, in both the investigational and control groups. These events included back and leg pain, neurological events, gastrointestinal events, spinal events, cardiovascular events and infection.

The rate of graft site related events was 8% in the control group and not applicable in the investigational groups.

[1] Percent of 140 males.
[2] Percent of 70 males.

Some of the reported adverse events required surgical interventions subsequent to the initial surgery. The number of subjects requiring a second surgical intervention was 10.4% (30/288) in the investigational groups and 13.7% (19/139) in the control group. The majority of supplemental fixations were due to painful nonunion.

Urogenital events occurred with greater frequency in the investigational groups (11.5%) compared to the control group (7%). Retrograde ejaculation rates were greater in the investigational groups (11 subjects) compared to the control group (1 subject) with the majority of events occurring in the early postoperative period.

The incidence of adverse events that were considered device related, including implant displacement/loosening, implant malposition and subsidence were all greater in the investigational groups compared to the control group. The rates of these events were low, however, and may be partially attributed to a learning curve associated with the laparoscopic surgical approach. The rate of nonunion requiring secondary surgery in the investigational groups was comparable to that of the control group. One death was reported - a control group subject with cardiovascular disease.

Potential Adverse Events:

The following is a list of potential adverse events which may occur with spinal fusion surgery with the InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device. Some of these adverse events may have been previously reported in the adverse events table.

- Bone fracture.
- Bowel or bladder problems.
- Cessation of any potential growth of the operated portion of the spine. Loss of spinal mobility or function.
- Change in mental status.
- Damage to blood vessels and cardiovascular system compromise.
- Damage to internal organs and connective tissue.
- Death.
- Development of respiratory problems.
- Disassembly, bending, breakage, loosening, and/or migration of components.
- Dural tears.
- Ectopic and/or exuberant bone formation.

- Fetal development complications.
- Foreign body (allergic) reaction.
- Gastrointestinal complications.
- Incisional complications.
- Infection.
- Insufflation complications.
- Neurological system compromise.
- Nonunion (or pseudarthrosis), delayed union, mal-union.
- Postoperative change in spinal curvature, loss of correction, height, and/or reduction.
- Retrograde ejaculation.
- Scar formation.
- Tissue or nerve damage.

Note: Additional surgery may be necessary to correct some of these potential adverse events.

IX. SUMMARY OF NONCLINICAL LABORATORY STUDIES

Three sets of nonclinical laboratory studies were preformed – those related to the LT-CAGE™ Lumbar Tapered Fusion Device component alone, those related to the InFUSE™ Bone Graft component alone, *i.e.*, rhBMP-2/ACS, and those related to the complete device, *i.e.*, the InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device.

IX.1 Characterization of the LT-CAGE™ Lumbar Tapered Fusion Device Component

The LT-CAGE™ Lumbar Tapered Fusion Device component was originally approved for use in the treatment of degenerative disc disease in the lumbar spine in P970015/S10. That PMA supplement, as well as the preclinical module for the current PMA, contained a description of the design characteristics and mechanical performance of this device component compared to that of the previously-approved parallel-sided spinal fusion cage (referred to as the INTER FIX™ Threaded Fusion Device). These data are summarized below.

IX.1.1 Design characteristics

The following table summarizes the design characteristics between a previously-approved design of the fusion cage component (the INTER FIX™ Threaded Fusion Device) and the version of the device utilized in the current combination device:

Characteristic	INTER FIX®	LT-CAGE®
Material	Ti-6Al-4V alloy (ASTM F-136 or ISO equivalent)	same
Shape	cylindrical (parallel sides)	tapered
Outer diameter (mm)	12 - 20	distal end: 14 – 18 proximal end: 18 –22
Length (mm)	20 – 29	20 – 26
Restored lordosis angle (°)	5	8
Surface porosity (%)	40 – 45	38 – 50
Hole to metal ratio (%)	35	33

IX.1.2 Mechanical Testing

The sponsor performed a number of mechanical tests to compare the mechanical behavior of the LT-CAGE™ Lumbar Tapered Fusion Device component to that of a previously-approved fusion cage design and, where appropriate, to the expected *in vivo* loads. These tests were performed under both static and dynamic conditions and were conducted in accordance with appropriate mechanical testing standards, *e.g.*, ASTM. The sponsor also performed tests which evaluated the impact of the LT-CAGE™ Lumbar Tapered Fusion Device component on the intrinsic stiffness of cadaveric spines. These results (averages) are summarized below:

Test	LT-CAGE®	INTER FIX®	Max. *in vivo* load
Static axial compression yield strength (N)	47,646	79,797	4000 – 13,000
Dynamic axial compression load at runout ($5x10^6$ cycles) (N)	at least 9600	at least 9600	2000 – 3000
Dynamic multi-axial compression load at runout ($5x10^6$ cycles) (N-m)	95	113	33
Static shear strength (N)	37,728	30,572	3200
Dynamic shear strength (N)	at least 5600	4000	1400
Insertion torque (N-m)	0.95	0.87	-
Pushout load (N)	711	697	-
Construct stiffness in calf spines (% of intact spine)	Flexion - 122.90 Extension - 127.24	Flexion - 71.17 Extension - 119.34	-

IX.2 Characterization of the InFUSE™ Bone Graft Component

The safety of rhBMP-2/ACS has been evaluated in an extensive series of toxicology studies of both the rhBMP-2 growth factor alone and the rhBMP-2 growth factor in combination with the ACS carrier/scaffold.

In the following sections, the text describes the general results from a class of studies, while the accompanying tables identify specific studies and their outcome.

IX.2.1 Biocompatibility Studies

The safety of rhBMP-2/ACS was evaluated in a series of biocompatibility tests. Under the conditions of these studies, there was no mortality or evidence of significant systemic toxicity in the mouse, no intracutaneous toxicity or significant dermal irritation in the

rabbit, no evidence of delayed dermal contact sensitization in the guinea pig, no evidence of cell lysis or toxicity in the extract and overlay cytotoxicity tests, no evidence of hemolysis, and no evidence of cellular mutagenicity. In the rabbit muscle irritation study, macroscopic evaluation of rhBMP-2/ACS revealed a hard, granular formation around the test site. The implant was graded macroscopically as a slight irritant relative to the negative control. Microscopically, the implant was graded as a nonirritant. The implant site revealed the presence of new bone formation consistent with the known pharmacologic action of rhBMP-2.

Study Type: Species	Groups/ No. Animals/ Sex	Route	Relevant Findings
Intracutaneous toxicity: rabbit: New Zealand white	1/2	IC	The test article extracts showed no evidence of causing significant irritation or toxicity.
Delayed contact sensitization: Guinea pig	2/15/F	ID skin patch	The test article extracts showed no evidence of causing sensitization.
Cytoxicity/ *in vitro*: WI-38 Human embryonic cell line	n/a	n/a	The test article extracts showed no evidence of causing cell lysis or toxicity.
Cytoxicity/ *in vitro* agarose overlay: L-929 mouse fibroblast cell line	n/a	n/a	The test article extracts showed no evidence of causing cell lysis or toxicity.
Systemic toxicity study/mouse	n/a	IV IP	The test article extracts were not considered systemically toxic to the mouse at the prescribed USP dosage.
In vitro hemolysis: rabbit whole blood	n/a	n/a	The test article extracts were not considered hemolytic.
Surgical muscle implantation study: rabbit/	n/a	IM	The test articles were considered to be trace-to-mild irritants after implantation in muscle.
Ames *in vitro* mutagenicity study	n/a	n/a	The test article extracts were not considered mutagenic.

n/a = not applicable

IX.2.1.2 Safety of rhBMP-2 Administered Intravenously: Acute and repeated dose, 28-Day Toxicity Studies

rhBMP-2 protein was studied in single- and multiple-dose general toxicology studies in the rat and beagle dog with up to 28 days of daily dosing. rhBMP-2 was administered intravenously (IV), at a range of doses that varied from slightly lower to substantially higher than the total doses (weight based) of rhBMP-2 that have been used in human clinical trials. There were no treatment-related toxicities observed in these studies. For example, although rhBMP-2 has potent bone-inducing activity at the local site of administration, systemic administration of suprapharmacologic doses of rhBMP-2 did not result in disseminated bone formation at any remote site, in any study.

Study Type: Species/ Strain	Groups/ No. Animals/ Sex	rhBMP-2 (mg/kg)	Relevant Findings
Acute toxicity: Rat/ Sprague-Dawley with sacrifices on Days 2, 7 and 15	5/ 5/sex sacrifice	saline vehicle 0.053 0.160 0.533	No toxicity observed. No-toxic-effect dose was 0.533 mg/kg IV.
Acute toxicity: Rat/ Sprague-Dawley with sacrifices on Days 2 and 15	4/ 5/sex sacrifice	vehicle 0.53 1.6 5.3	No treatment-related findings in animals sacrificed at Day 2. Slight-to-mild dose-related chondrogenesis at injection sites. No-toxic-effect dose was 5.3 mg/kg IV.
Acute toxicity: Dog/ Beagle Sacrifice on Day 15	4/ 1/sex	vehicle 0.53 1.6 5.3	No toxicity observed. No-toxic-effect dose was 5.3 mg/kg IV.
28-Day toxicity: Rat/ Sprague-Dawley	5/ 10/(5)[a]/sex	saline vehicle 0.016 0.05 0.16	Ten deaths unrelated to treatment (vehicle, 0.016, 0.05, 0.16) Dose-related soft tissue thickening and cartilage formation in subcutaneous tissue at injection sites. Following 28-day recovery period, the soft tissue thickening regressed and matured to bone. No-toxic-effect dose was 0.16 mg/kg/day IV.
28-Day toxicity: Dog/ Beagle	5 3/(2) [a] /sex	saline vehicle 0.016 0.05 0.16	Dose-related perivascular fibroplasia at injection site in all rhBMP-2-treated animals with bone formation in mid- to high-dose groups. No-toxic-effect dose was 0.16 mg/kg/day IV.

[a] () numbers of additional recovery sub-group animals in control and high-dose groups

IX.2.1.3 Chronic Toxicity

The long-term safety of implanted rhBMP-2/ACS was evaluated in two studies, a 6-month mandibular/maxillofacial inlay study in beagle dogs and a 1-year femoral onlay study in Sprague-Dawley rats. These studies were designed to assess the potential long-term systemic and local effects of rhBMP-2/ACS in two species at two skeletal sites. Implants of rhBMP-2/ACS had no systemic effects and local effects were associated with the osteoinductive activity of rhBMP-2. Transient, low-titer immune responses were observed in the dog study.

Study Type: Species/ Strain	Groups/ No. Animals/ Sex	rhBMP-2 (mg/kg)	Relevant Findings
6-month Mandibular/ Maxillofacial Implant (at inlay defect site): Dog/Beagle	5 2/sex sacrificed at 3 and 6 months post-implantation	sham surgery, vehicle/ACS, 0.078 mg/kg (0.4 mg/mL)/ACS, 0.312 mg/kg (1.6 mg/mL)/ACS, 0.781 mg/kg, (4.0 mg/mL)/ACS	No effects of treatment on clinical signs, hematology, or clinical chemistry. Dose-related post-surgical swelling. As swelling subsided (3-4 weeks), firm masses near the zygomatic and mandibular implant sites were detected in most rhBMP-2-treated animals. Histologically, the rhBMP-2-treated implant sites were composed of abundant fibrocellular tissue and/or new bone formation within and around the defect site. There were fluid filled tissue cysts and occasionally strands of residual ACS material at implant sites with apparent regression between 3 and 6 months. Implant site changes were expected exaggerated pharmacologic responses to rhBMP-2/ACS and were not toxicologically significant. No-toxic-effect dose was 0.781 mg/kg (4.0 mg/mL concentration rhBMP-2). Transient low titer antibody responses were observed in 15/24 (62.5%) of the treated animals.
1-year Femoral onlay Implant Toxicology: Rat/Sprague-Dawley	5 10/sex sacrificed at 1, 6, and 12 months post-implantation	vehicle/ACS, 0.04 mg/kg (0.1 mg/mL)/ ACS, 0.3 mg/kg (0.75 mg/mL)/ ACS, 1.6 mg/kg (4.0 mg/mL)/ ACS	Slight increased incidence and severity of surgical site swelling at 1.6 mg/kg. Dose-related pharmacologic effect of increased incidence and/or severity of bone formation at implant site in all rhBMP-2/ACS treatment groups. No toxicity at any dose.

IX.2.1.4 Safety of rhBMP-2 Administered Intravenously: Fertility, General Reproductive Performance and Teratology

Because BMP-2 participates in embryological development, rhBMP-2 was evaluated for any effect on reproduction or fetal development. Studies evaluated rhBMP-2 at total doses (weight based) that ranged from slightly lower to substantially higher than rhBMP-2 doses that are anticipated in clinical use (up to approximately 1 mg rhBMP-2/kg, total delivered dose). The effects of rhBMP-2 on the reproduction and fertility of male and female Sprague-Dawley rats was studied. Maternal and paternal mating performance and reproductive parameters were not affected by treatment. Range-finding studies followed by developmental toxicity studies were conducted in both Sprague-Dawley rats and New Zealand white rabbits. There was no evidence of maternal toxicity, embryolethality, fetotoxicity, or teratogenicity.

Study Type: Species/ Strain	Groups/ No. Animals/ Sex	rhBMP-2 (mg/kg)	Relevant Findings
Fertility: rat/Sprague-Dawley	5/ 40/F 40/M	saline vehicle 0.016 0.05 0.16	Maternal and paternal mating performance and reproductive parameters were not affected by treatment. No-toxic-effect dose was 0.16 mg/kg/day IV.
Range-finding Teratology: rabbit/ New Zealand white rabbit	7/ 5/F	saline vehicle 0.016 0.05 0.16 0.5 1.6 Days 6 to 18 gestation	No maternal toxicity, embryolethality, or gross fetal abnormalities. No-toxic-effect level was 1.6 mg/kg/day IV.
Teratology: rabbit/New Zealand white	5/ 20/F	saline vehicle 0.016 0.5 1.6 Days 6 to 18 gestation	No maternal toxicity, embryolethality, or gross fetal abnormalities. No-toxic-effect level was 1.6 mg/kg/day IV. Definitive teratology study in rats. The incidences of malformations were not significantly different between control and treated groups.
Range-finding Teratology: Rat/ Sprague-Dawley	7/ 6/F	saline vehicle 0.016 0.05 0.16 0.5 1.6 Days 6 to 17 gestation	No maternal toxicity, embryolethality, or gross fetal abnormalities. No-toxic-effect level was 1.6 mg/kg/day IV.
Teratology: Rat/ Sprague-Dawley	5/ 25/F	saline vehicle 0.16 0.5 1.6 Days 6 to 17 gestation	No maternal toxicity, embryolethality or fetal abnormalities. No-toxic-effect dose was 1.6 mg/kg/day IV. The definitive teratology study in rats was repeated. In the initial study there was a nonsignificant difference in skeletal formation between the rhBMP-2 groups and the saline and control groups. Examination of skeletal variance revealed a significant reduction in sternebral variance in all treated groups.

Study Type: Species/ Strain	Groups/ No. Animals/ Sex	rhBMP-2 (mg/kg)	Relevant Findings
Repeat teratology: rat/Sprague-Dawley	2/ 25/F	vehicle 1.6 Days 6 to 17 gestation	It was hypothesized that the difference in skeletal formation in the preceding study was a result of the time of cesarean section rather than a treatment effect. This repeat study had a random order of selection for time of cesarean section. No maternal toxicity, embryolethality, fetotoxicity or teratogenicity and no difference in skeletal formation between the control and rhBMP-2 groups. No-toxic-effect level was 1.6 mg/kg/day IV.

IX.2.1.5 Carcinogenicity/Genotoxicity

In addition to the Ames Mutagenicity assay, the sponsor investigated the potential for rhBMP-2 to stimulate the proliferation of primary tumor cell isolates and tumor cell lines. rhBMP-2 was examined for growth potentiating activity *in vitro* on human tumor cell lines and primary tumor cell isolates at concentrations of 10 to 1000 ng/mL. No growth potentiating activity was observed. rhBMP-2 exhibited growth inhibition of several carcinoma-derived tumors. The studies did not include analyses to determine if the cells expressed BMP type I and II receptors. Overall, studies investigating the potential effects of rhBMP-2 on tumor cell growth showed minimal evidence of growth potentiation including studies of osteosarcoma cell lines.

Study Type: Species/ Strain	Groups/ No. Animals/ Sex	rhBMP-2 (mg/kg)/Route	Relevant Findings
Growth potential on primary tumor isolates *in vitro* (Soda et al., *Anti-Cancer Drugs*, 1998)	n/a	10, 100, and 1000 ng/mL concentration *in vitro*	No tumor cell growth stimulation. Significant inhibition of colony forming units in 16 of 65 specimens at 1000 ng/kg
Inhibition of tumor growth *in vitro* with human tumor cell lines	n/a	10, 100, and 1000 ng/mL concentration *in vitro*	No effect on osteosarcoma cell line growth. Inhibitory effects on several soft tissue carcinoma cell lines.

IX.2.2 Immunology

Formation of antibodies to rhBMP-2 and Type I collagen in canines, rhesus monkeys and rats was monitored using Enzyme Linked Immusorbent Assays (ELISAs). In general, rhBMP-2 or bovine Type I collagen was coated onto microtiter plates. Controls or serum samples were diluted in assay buffer and incubated on the plates. Enzyme-conjugated reagents were used to detect bound antibodies. Enzyme substrates were then incubated on the plates and optical densities of the solutions were measured in order to quantitate

the presence of anti-rhMBP-2 or Type I collagen antibodies. Immune responses to rhBMP-2 were observed in nonhuman primates and in dogs. Please refer to the 6 month beagle dog study in section IX.2.1.3, Chronic Toxicity, and the radius, critical size defect repair/nonhuman primate study in section IX.2.5, Animal Studies.

IX.2.3 Pharmacokinetics

IX.2.3.1 rhBMP-2 Protein Administered Intravenously

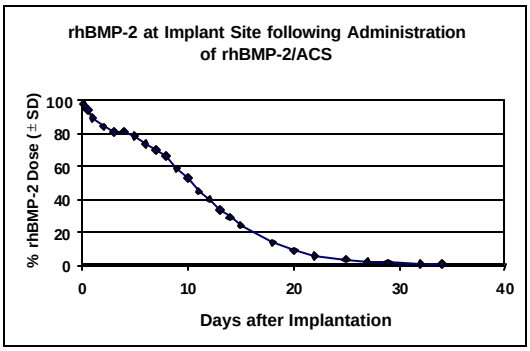
Although rhBMP-2 is intended to be delivered with the ACS as two part device component, pharmacokinetic results obtained from IV dosing provide a means to evaluate the extent and duration of systemic exposure of rhBMP-2. Studies were conducted to characterize the pharmacokinetics of rhBMP-2 in the blood of rats and monkeys. The uptake of rhBMP-2 by highly perfused tissues and organs is rapid, but the residence of the protein is short. Catabolism of the protein is extensive and renal excretion of trichloroacetic acid-soluble radioactivity is rapid. rhBMP-2 is rapidly eliminated in rat and nonhuman primates ($t_{½}$ = 16 minutes in the rat and $t_{½}$ = 6.7 minutes in nonhuman primates) from the systemic circulation following intravenous administration. A study conducted in juvenile and adult Sprague-Dawley rats revealed that juvenile rats, like adult rats, cleared rhBMP-2 rapidly. Results also showed a lower maximal concentration, higher clearance and a larger initial volume of distribution for rhBMP-2 in juvenile rats as compared to adult rats. As a result of these pharmacokinetic characteristics, systemic presence of rhBMP-2 in the circulation is minimal after IV dosing.

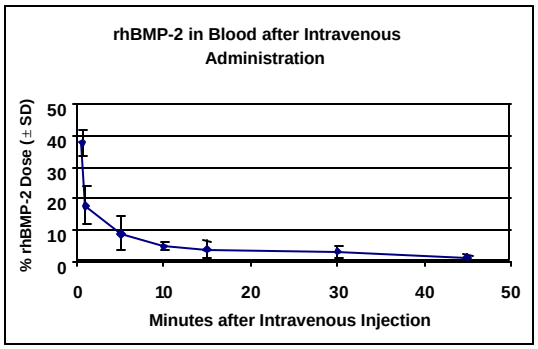
Study Type/ Species/ Strain	Groups/ No. Animals (per group)	rhBMP-2 Dose	Relevant Findings
PK single dose: rat/Sprague-Dawley	4/3	0.43 4.3 43 860 µg/kg	Clearance of ^{125}I-rhBMP-2 was rapid and biexponential: $T_{1/2\alpha}$[a] = 0.8 min, and $T_{1/2\beta}$[b] =15.3 min. Most of the administered dose (92%) was recovered by 24 hours in the urine as TCA-soluble counts per minute. [a] $T_{1/2\alpha}$ = half-life of initial phase [b] $T_{1/2\beta}$ = half-life of terminal phase
PK single dose: nonhuman primate/ cynomolgus monkey	2/3	4.9 µg/kg	Clearance of ^{125}I-rhBMP-2 was rapid and biexponential: $T_{1/2\alpha}$[a] = 1.0 min, and $T_{1/2\beta}$[b] =7.0 min.
Biodistribution: rat/Sprague-Dawley	8/3	4.3 µg/kg	Rapid localization to liver with metabolism and excretion into urine. Biphasic disposition was observed with initial and terminal half-life of 0.8 and 31 minutes, respectively.
Biodistribution: rat/Sprague-Dawley	8/3	7.1 µg/kg	^{125}I-rhBMP-2 rapidly distributed to the highly perfused tissues; 1 minute after dosing, 82.4% of the dose was recovered in the liver, lung, kidney, and spleen. The liver was the predominant site of ^{125}I-rhBMP-2 localization throughout the study.
PK single dose: juvenile and adult rats/Sprague-Dawley	2/12-24	3.0 mg/kg	Clearance of ^{131}I-rhBMP-2 was rapid and biexponential in both juvenile and adult rats as assessed by serum acid precipitable radioactivity and by ELISA.

IX.2.3.2 Local Retention of rhBMP-2 Administered With ACS

The local residence time of rhBMP-2 when applied to the ACS was assessed following subcutaneous (SC) implantation in rats and implantation at orthotopic sites in rats and rabbits. The results from all three models were similar. In the rat femoral onlay model, ^{125}I-rhBMP-2 was slowly released from the implant site with a mean residence time of approximately 8 days (refer to Figure below). The peak amount of radiolabeled rhBMP-2 detected in the blood was small, 0.1% of the implanted dose, and consistent with the rapid systemic clearance described above.

Retention of rhBMP-2 Following Implantation of rhBMP-2/ACS (left) and Pharmacokinetic Evaluation Following Intravenous Administration of rhBMP-2 (right) in the Rat





IX.2.3.3 Interactions Between rhBMP-2 and the Absorbable Collagen Sponge

The importance of retaining rhBMP-2 at the implant site for optimal bone formation was highlighted in one study in which the heparin-binding domain of rhBMP-2 was removed using plasmin. Following implantation with ACS, this modified protein was found to leave the implant site much more rapidly than the native protein; for example, only 18% of the plasmin-cleaved material was present at 3 hours, contrasting with 56% for the native protein. Though the plasmin-cleaved protein was highly active in a cell culture assay, bone formation *in vivo* was substantially reduced, indicating that retention of bioactive material at the implant site is critical for the desired osteogenic activity.

As the local retention of rhBMP-2 is important for localized bone formation, several studies evaluated the effect of potential variables on retention following SC implantation in the rat or orthotopic implantation in the rabbit. The relative retention of rhBMP-2 was unaffected by the concentration of rhBMP-2 administered (between 0.8 and 2.0 mg/mL) or the formulation buffer. The amount of rhBMP-2 incorporated into ACS (a measure of the binding of rhBMP-2 to the sponge prior to implantation) had a minimal effect on rhBMP-2 retention *in vivo*, and no effect on the rate of release of rhBMP-2 into serum *in vitro*. These results suggest that the release of rhBMP-2 *in vivo* is independent of the binding of rhBMP-2 to the sponge *in vitro*, and may be diffusion-controlled.

IX.2.4 Effect of Nicotine and Glucocorticosteroids on Implantation with rhBMP-2/ACS

In animal studies, the sponsor has shown that rhBMP-2/ACS induces bone and fracture repair in the presence of several agents which compromise bone metabolism, for example, nicotine and corticosteroids.

Study Type/ Species	Groups/ No. Animals	rhBMP-2 (mg/mL)	Relevant Findings
Subcutaneous implant/rat	8[a]/4-5	0 0.01 0.1 0.4	Systemic nicotine treatment did not inhibit the ability of rhBMP-2/ACS to induce bone formation.
Ulnar osteotomy repair/rabbit	3[b]/12-13	0.2	Nicotine did not affect the rate of fracture repair in this model.
Subcutaneous implant/rat	8[c]/6	0 0.01 0.1 0.4	Prednisolone treatment dramatically inhibited bone growth and body mass gain. Prednisolone treatment also inhibited ectopic bone formation; however, the high dose of rhBMP-2/ACS overcame this inhibition.
Ulnar osteotomy repair/rabbit	4[d]/12	0 0.2	Prednisolone treatment inhibited fracture healing. Treatment with rhBMP-2/ACS overcame this inhibition and enhanced healing in both the control and prednisolone treated animals.

[a] four nicotine-treated; four control
[b] nicotine-treated; nicotine pre-treated, or control
[c] four prednisolone-treated; four control
[d] prednisolone-treated or control; two sacrifice timepoints

IX.2.5 Animal Studies

Pharmacology studies have demonstrated that rhBMP-2/ACS can induce bone and repair large, segmental critical-sized defects in rat femora, rabbit radii and ulnae, dog radii, and nonhuman primate radii. The induced bone biologically and structurally integrates with the pre-existing bone, and remodels physiologically, *i.e.*, consistent with the biomechanical forces placed on it. In addition, the rhBMP-2/ACS-induced bone can repair itself following fracture, in a manner indistinguishable from native bone. Separate studies demonstrated that rhBMP-2/ACS can accelerate healing in rabbit and goat long bone fracture models. Thus, radiographic, biomechanical, and histologic evaluation of the induced bone indicates that it is appropriate for the anatomic site where it forms, and functions biologically and biomechanically as native bone.

Histologic analyses from many pharmacology studies have characterized the cellular events involved in the bone induction process initiated by rhBMP-2/ACS. Mesenchymal cells from the surrounding tissues first infiltrate the periphery of the rhBMP-2/ACS implant. As the ACS is degraded, these cells appear to differentiate and begin to form trabecular bone and/or cartilage. Vascular invasion is evident at the same time. The bone formation process temporally extends from the periphery of the rhBMP-2/ACS implant towards the center, until the entire rhBMP-2/ACS implant is replaced by trabecular bone. Remodeling of the trabecular bone then occurs depending on the physiologic form of the site and function applied to the bone. This ability of rhBMP-2/ACS to support bone remodeling may also be responsible in part for the biologic and biomechanical integration of the new bone induced by rhBMP-2/ACS with that of the surrounding bone.

Study Type/ Species	Groups/No. Animals (per group)	rhBMP-2 (mg/mL)	Relevant Findings
Subcutaneous implant/rat	5/6	0 0.17 0.33 0.66 1.7	Substantial bone induction was observed in all implants containing rhBMP-2.
Femur critical sized defect repair/rat	6/7	0.013 0.025 0.05 0.1 0.2 0.4	Dose-response-related healing of critical-sized bone defects measured by radiologic and histologic criteria. Therapeutic dose (concentration) range of 0.025 – 0.05 mg/mL rhBMP-2/ACS delineated for this model.
Radius critical-sized defect repair/dog	4/3	0 0.05 0.2 0.8	All rhBMP-2/ACS-treated animals healed at 12 weeks by all criteria. Dose-dependent generation of excess bone and voids; voids observed especially in bone formed outside of implant area. Biomechanical values of all rhBMP-2 dose groups are equal or superior to autologous bone-grafted controls.
Radius critical-size defect repair/dog	3/5	0 0.05 0.2	Functional loading of rhBMP-2/ACS-treated radii at 16 week demonstrated. Remodeling and consolidation of induced bone observed during subsequent 8-32 week. Remodeling over time of void spaces observed. Stress fractures in rhBMP-2/ACS and autogenous bone grafted limbs occurred at similar frequencies and healed at similar rates.
Radius critical-size defect repair/nonhuman primate	9/2-6	0 0.05 0.2 0.4 0.8 1.3 1.5 1.9 3.1	Variable bone induction observed in range of 0.4 to 1.5 mg/ml rhBMP-2/ACS. No dose-response defined. No radiolucent voids generated at any dose. Dose-related generation of antibodies to rhBMP-2 were observed in 35% (7/20) treated. Antibodies to Type I collage were observed in 7% (1/14); no correlation with efficacy.
Ulnar critical-size defect repair/nonhuman primate	6[a]/1	0 0.75	Bone induction within defect by 4 weeks. Accelerated removal of ACS in presence of rhBMP-2. Compression of ACS limited amount of bone induction observed.

Study Type/ Species	Groups/No. Animals (per group)	rhBMP-2 (mg/mL)	Relevant Findings
Femoral head core defect/sheep	3[a]/2	0 0.4 1.5	Superior retention of rhBMP-2, bone induction within defect and bone formation surrounding defect when implanted with ACS as compared to injection in buffer. rhBMP-2/ACS induced early remodeling in trabecular bone surrounding implantation site.
Femoral head core defect/sheep	2[b]/2	0 0.8 1.5	Superior bone induction observed in response to rhBMP-2/ACS compared to ACS alone or surgical control. Early bone resorption surrounding defect observed followed by subsequent bone formation. A positive correlation was found between retention of rhBMP-2 at the implant site and bone formation.
Ulnar osteotomy repair/rabbit Pilot I[b] Pilot II[b] Main	many/5-15 3[a]/10 2[c]/15 6/15	0 0.05 0.1 0.2 0.4 0.8	Increase in torsional loading parameters with rhBMP-2/ACS placed as an onlay. rhBMP-2/ACS-treated limbs healed at 3-4 weeks, as compared to 6 weeks in controls.
Ulnar osteotomy repair/rabbit	2/6[d]	0 0.2	Torsional biomechanics in the rhBMP-2/ACS group 80-100% greater than surgical control and ACS groups at 3 and 4 weeks. No difference between surgical and ACS onlay groups at any time point. Approximately 25% decrease in time to bony union with rhBMP-2/ACS.
Tibial fracture repair/goat	2/1-2	0.2 0.8	No obvious differences in treated and control fractures. Conclusions difficult to reach due to small sample size and lack of carrier control groups.
Tibial fracture repair/goat	2/4	0 0.4	rhBMP-2/ACS used as an onlay resulted in increased callus size and maturity, as assessed radiographically and histologically. Fractures wrapped with rhBMP-2/ACS had increased torsional toughness.
Femoral allograft incorporation/dog	3/7	0 0.4	Augmentation of the allograft-host bone junctions with rhBMP-2/ACS resulted in a stronger and more complete union than with autogenous bone graft or ACS alone.
Radius defect repair/rabbit	4/8	0 0.1 0.4	No deleterious effects on radiocarpal joint observed when rhBMP-2/ACS placed in a distal radius defect

Study Type/ Species	Groups/No. Animals (per group)	rhBMP-2 (mg/mL)	Relevant Findings
		1.3	connected to joint cavity.
Mid-diaphyseal Ulnar Osteotomy repair/rabbit	4[e]/6	0.4	Histologically there was no treatment effect on morphology of the growth plates.
Ulnar osteotomy repair/rabbit	7[f]/3-9	0.2	As assessed by radiography and histology, defects in bone induced by rhBMP-2/ACS heal by a process similar to that seen in normal bone.

[a] sacrifice at three timepoints
[b] contralateral control = ACS or not treatment
[c] sacrifice at two timepoints
[d] at each of four timepoints
[e] two or three month old rabbits; rhBMP-2/ACS or surgical control
[f] seven timepoints

IX.2.6 Preclinical Effectiveness Evaluations Conclusions

Many of the animal pharmacology studies have included dose-ranging, and from these results, a broad therapeutic concentration range, measured as quantity of rhBMP-2 per unit volume of ACS, has been determined. This therapeutic range is bordered on one side by inadequate bone formation and on the other by excessive bone formation combined with an increased incidence and size of fluid-filled void spaces within the induced bone (both of which remodeled appropriately over time). The therapeutic rhBMP-2 concentration range shifts with the animal species tested in apparent accord with the bone formation rate of that animal. Thus, higher concentrations are required in dogs than in rats, and even higher concentrations in nonhuman primates. The upper end of the therapeutic concentration range in nonhuman primates has not been defined by these studies. The nonhuman primate therapeutic range, 0.4 – 1.5 mg/mL rhBMP-2, is the same that has been tested in humans. Both the concentration of rhBMP-2 and the length of time that rhBMP-2 is present at the implant site are positively correlated with the rate of bone formation, the amount of bone formed, and the density of the resulting bone.

In summary, the safety (toxicology and pharmacokinetics) and bone-forming capacity of rhBMP-2/ACS have been extensively investigated and are well understood. The nonclinical safety of systemically delivered rhBMP-2 and locally delivered rhBMP-2/ACS has been extensively studied and no toxicities have been identified in these studies. The disposition of rhBMP-2 and rhBMP-2/ACS is characterized by slow release of rhBMP-2 from the implantation site and rapid systemic clearance. This profile results in minimal systemic exposure to rhBMP-2. Application of rhBMP-2/ACS results in the induction of normal bone locally at the site of implantation. This process includes the migration of mesenchymal cells into the site, their proliferation and apparent differentiation into bone-forming cells. The bone induced by rhBMP-2/ACS remodels and assumes the structure appropriate to its location and function, as would be expected from host bone.

IX.2.7 Characterization of the InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device

IX.2.7.1 Preclinical Safety Studies

The studies listed below were performed to ensure that the rhBMP-2/ACS was safe for use in proximity to the spinal cord and to identify a dose of rhBMP-2 sufficient to cause bone formation. The purpose of the first study was to assess the effects of a rhBMP-2/ ACS combination on exposed dura and neural tissue after standard decompressive lumbar laminectomy using a canine model. This study represented a worst case situation in which rhBMP-2/ACS or autogenous bone graft was in direct contact with the spinal cord. The following three studies assessed rhBMP-2/ACS induced fusion rates in monkeys and dogs.

Study Type	Species (n/ group)/Device(s) tested	rhBMP-2/ACS mg/site (mg/mL)	Relevant Findings
Implantation on exposed dura after laminectomy	Dog/ RhBMP-2/ACS	0.24 (0.10)	Clinical observation, radiography, CT scans, neurological exam, histology: No neurological deficit, no spinal cord stenosis and no mineralization of the dura when rhBMP-2 placed directly on exposed dura. There was no difference between animals that received a dural nick and those that did not.
Lumbar: dosing study	monkey (2)/ 1. rhBMP-2/ACS 2. rhBMP-2 Open Porosity Polylactic Acid Sponge (OPLA) 3. Autograft	2 (0.43) 4 (0.85) 8 (1.70)	Clinical evaluation, radiography, CT scans, histology: Highest dose had equal fusion rate to autograft of 50%. The two lower doses did not result in fusions.
Lumbar: dosing study	monkey (2)/ rhBMP-2/ACS	6 (0.43) 18 (1.29) 32 (2.27)	Clinical evaluation, radiography, CT scans, histology: Significantly larger amounts of bone was formed in this study but inconsistently. Only 18 mg treated animals and one of 32 mg dose fused.
Lumbar: dosing study	dog (2-4)/ rhBMP-2/OPLA	0 (0) 0.06 (0.025) 0.11 (0.05) 0.23 (0.10) 0.46 (0.20) (0.92) (0.40)	Clinical evaluation, radiography, CT scans, histology, biomechanical testing: All dogs treated with rhBMP-2 fused at 12 weeks as assessed by evaluation criteria. No statistical difference in fusion rate vs. dosage.

IX.2.7.2 Preclinical Efficacy Studies Directly Supportive of Lumbar Spinal Fusion Intended Use

The efficacy studies listed below are directly relevant to the intended clinical indication and are presented as primary data for the preclinical efficacy studies of rhBMP-2. These studies were performed to investigate the effectiveness of rhBMP-2/ACS in promoting interbody fusion of the lumbar spine. In three of these studies, the efficacy of rhBMP-

2/ACS was compared to that of autogenous bone graft. The fourth study compared the ACS carrier alone to the ACS carrier with one of two different doses of rhBMP-2.

Study Type	Species (n/ group)/Device(s) tested	rhBMP-2/ACS mg/site (mg/mL)	Relevant Findings
Laproscopic anterior interbody fusion using titanium cage	monkey (2-3)/ rhBMP-2/ACS/titanium cage	0 (0) 0.34 (0.75) 0.68 (1.50)	Clinical evaluation, radiography, CT scans, histology: All monkeys treated with rhBMP-2 fused by 24 weeks. Neither of animals without rhBMP-2 fused.
Anterior interbody using titanium cage	sheep (6)/ 1. rhBMP-2/ACS/allograft bone dowel 2. autograft/titanium cage	0.69 (0.43)	Clinical evaluation, radiography, CT scans, histology, biomechanical testing: All sheep treated with rhBMP-2 fused at 24 weeks as assessed by histological evaluation. 37% of autografts fused.
Anterior interbody	monkey (3)/ 1. rhBMP-2/ACS/allograft 2. Autograft bone dowel	0.40 (1.50)	Clinical evaluation, radiography, CT scans, histology: All monkeys tested with rhBMP-2 fused by 24 weeks. 50% of autograft controls were fused at 24 weeks.
Lumbar interbody	sheep (6)/ 1. rhBMP-2/ACS/Carbon fiber reinforced polymer device 2.Autograft/carbon fiber reinforced polymer device	0.43 (0.43)	Clinical evaluation, radiography, histology: 86% of rhBMP-2 treated animals fused histologically vs. 83% of autograft controls. No difference in biomechanical testing.

IX.2.7.3 Posterolateral/Other Surgical Techniques

The efficacy studies listed below are presented as supporting information which are relevant to the intended clinical use. These studies were performed in order to gain information on the use of rhBMP-2/ACS in the lumbar spine using different surgical techniques.

Study Type	Species (n/ group)/Device(s) tested	rhBMP-2/ACS mg/site (mg/mL)	Relevant Findings
Lumbar with and without protective polyethylene shield	Monkey (2)/ 1. rhBMP-2/ACS 2. rhBMP-2/ACS + shield	9 (1.5) 18 (1.5)	Clinical evaluation, radiography, CT scans, and histology: All monkeys treated with rhBMP-2 fused at 16 weeks as assessed by evaluation criteria. Animals with protective shield over fusion mass had more robust fusions despite lower dose of rhBMP-2
Lumbar	rabbit (4-16)/ 1. rhBMP-2/ACS 2. autograft	0 (0) 2.7 (0.64)	Clinical evaluation, radiography, CT scans, and histology: All rabbits treated with rhBMP-2 fused at 4 weeks as assessed by evaluation criteria. 42% of autografts fused.
Open vs. minimally invasive technique	rabbit (16)/ rhBMP-2/ACS	0.24 (0.1)	Clinical evaluation, radiography, CT scans, and histology: All rabbits treated with rhBMP-2 fused at 10 weeks as assessed by evaluation criteria.
Lumbar	monkey (2-6)/ 1. rhBMP-2/BCP 2.Autograft	6.0 (1.36) 9.0 (2.04) 12.0 (2.72)	Clinical evaluation, radiography, CT scans and histology: All monkeys treated with rhBMP-2 fused at 24 weeks. No autograft treated animals fused.
Lumbar: Comparison of calcium phosphate carriers	monkey (2)/ 1. rhBMP-2/BCP 2. rhBMP-2/TCP 3. rhBMP-2/TCP + collagen	9.0 (2.04) 9.0 (1.50) 4.5 (0.75)	Clinical evaluation, radiography, CT scans and histology: All monkeys treated with rhBMP-2/BCP and TCP fused at 24 weeks.
Percutaneous technique	monkey (5)/ rhBMP-2/BCP	9.0 (2.04)	Clinical evaluation, radiography, CT scans and histology: All monkeys treated with rhBMP-2 fused at 24 weeks. No autograft treated animals fused.
Lumbar	rabbit (11)/ rhBMP-2/BCP	0.90 (0.43)	Clinical evaluation, radiography, CT scans and histology: The rhBMP-2/BCP fusion masses were stiffer and stronger than autograft fusion mass controls.
Lumbar	dog (6)/ 1. rhBMP-2/OPLA 2. Autograft	2.3/1.04	Clinical evaluation, radiography, CT scans, histology and biomechanical testing: All dogs treated with rhBMP-2 fused at 12 weeks as assessed by evaluation criteria. 8% of autografts fused.
Lumbar: rhBMP-2 and steroid study	rabbit (12)/ 1. rhBMP-2 2. Autograft	1.0 (0.67)	Clinical evaluation, radiography, and biomechanical testing: 89% of rabbits treated with rhBMP-2 fused despite treatment with a steroid. None of the rabbits without rhBMP-2 fused when treated with a steroid.

Study Type	Species (n/ group)/Device(s) tested	rhBMP-2/ACS mg/site (mg/mL)	Relevant Findings
Clinical Ortho Rel Res publication/ Posterior fusion with plating	dog (11) 1. Autograft 2. PGLA particles/blood clots 3. rhBMP-2/PGLA particles/blood clots	0 (0) 0.4 (0.2)	Clinical evaluation, radiography, histology and biomechanical testing: Dogs treated with rhBMP-2 had equal fusion rates to autograft.

IX.2.7.4 Additional Information

The efficacy studies identified in the first table represent additional information related to the use of the rhBMP-2 and ACS or other carriers in the lumbar spine. These data may be considered ambiguous, uninterpretable, or inconclusive, however, due to factors such as small sample sizes, no controls, etc.

Study Type	Species (n/ group)/Device(s) tested	rhBMP-2/ACS mg/site (mg/mL)	Relevant Findings
Interbody fusion studiess			
Lumbar	rabbit (2)/ 1. rhBMP-2/ACS 2. rhBMP-2/OPLA	0.48 (0.2) 0.96 (0.4)	Clinical evaluation, radiography, CT scans, and histology: All rabbits treated with rhBMP-2 fused at 4 weeks as assessed by evaluation criteria.
Lumbar	dog (3)/ 1. rhBMP-2/ACS 2. rhBMP-2/OPLA 3. Autograft	0 (0) 0.054 (0.023) 0.215 (0.09) 0.860 (0.36)	Clinical evaluation, radiography, CT scans, and histology: All dogs treated with rhBMP-2 fused at 12 weeks as assessed by evaluation criteria. 33% of autografts fused.
Lumbar without decortication	dog (3)/ 1.rhBMP-2/ACS 2. rhBMP-2/OPLA	0.06 (0.025) 0.23 (0.10) 0.92 (0.40)	Clinical evaluation, radiography, CT scans, histology and biomechanical testing: All dogs treated with rhBMP-2 fused at 12 weeks as assessed by evaluation criteria. No statistical difference seen in carriers.
Lumbar: Augmentation of autograft with rhBMP-2	dog (3)/ 1. Autograft 2. rhBMP-2/Autograft 3. rhBMP-2/ACS/Autograft 4. rhBMP-2/ACS 5. rhBMP-2/OPLA 6. rhBMP-2/Polylactic Glycolic Acid Sponge (PGLA)/Autograft	0.43 (0.43)	Clinical evaluation, radiography, CT scans, histology and biomechanical testing: Dogs treated with rhBMP-2 had significantly increased volumes of fusion mass compared to autograft. No difference in carriers.
Lumbar	monkey (1)/ 1. rhBMP-2/BCP 2. rhBMP-2/BCP/collagen 3. rhBMP-2/ collagen	6.0 (0.84) 18.0 (1.68) 18.0 (2.53)	Clinical evaluation, radiography, CT scans and histology: All monkeys treated with rhBMP-2 fused at 24 weeks.
Lumbar: Decortification vs. no decortication	dog (3-4)/ undecorticated rhBMP-2/OPLA	0.06 (0.025) 0.23 (0.10) 0.92 (0.40)	Clinical evaluation, radiography, CT scans, histology and biomechanical testing: All dogs treated with rhBMP-2 fused at 12 weeks as assessed by evaluation criteria except for one undecorticated low dose. No statistical difference in decorticated vs. undecorticated.

Study Type	Species (n/ group)/Device(s) tested	rhBMP-2/ACS mg/site (mg/mL)	Relevant Findings
Lumbar: rhBMP-2 and NSAID study	rabbit (9-17)/ 1. rhBMP-2 2. Autograft	3 (1.0)	Clinical evaluation, radiography, and histology: All rabbits treated with rhBMP-2 fused despite treatment with an NSAID. 75% of autografts fused without NSAID treatment. 35% of autografts with NSAID treatment fused.
Posterolateral fusion studies			
rhBMP-2/OPLA Pilot study	dog (1-5)/ 1. rhBMP-2/OPLA 2. rhBMP-2/OPLA + hyaluronic acid	0 (0) 0.67 (1.04)	Clinical evaluation, radiography and histology: All dogs treated with rhBMP-2 fused at 12 weeks as assessed by evaluation criteria.
Lumbar: Augmentation of autograft with rhBMP-2	dog (2)/ 1. Autograft 2.Autograft/ demineralized bone 3. Autograft/collagen 4.Autograft/ demineralized bone/collagen 5. rhBMP-2/Autograft	0 (0) 2.0 (0.34)	Clinical evaluation, radiography, CT scans, histology and biomechanical testing: Dogs treated with rhBMP-2 significantly increased the number of fused levels compared to the controls at 24 weeks. In addition, the fusion masses were larger with rhBMP-2.

IX.2.7.5 Studies that demonstrate the ability of rhBMP-2 to fuse other bone defects

The efficacy studies listed in the following table represent the use of rhBMP-2/ACS in the goat cervical spine model. All three of the studies compared the efficacy of rhBMP-2/ACS to that of autogenous bone graft.

Study Type	Species (n/ group)/Device(s) tested	rhBMP-2/ACS mg/site (mg/mL)	Relevant Findings
Interbody fusion	goat (7)/ 1. rhBMP-2/ACS/titanium cage 2. Autograft/titanium cage	0.20 (0.40)	Clinical evaluation, radiography, CT scans, histology and biomechanical testing: 95% of goats treated with rhBMP-2 fused at 12 weeks as assessed by histological evaluation. 48% of autografts fused.
Interbody fusion	goat (8) 1. rhBMP-2/ACS/titanium cage 2. Autograft/titanium cage 3. tantalum cage 4. rhBMP-2 tantalum cage	0.20 (0.40)	Radiography, CT scans, histology and biomechanical testing: 67% of levels treated with rhBMP-2 fused at 12 weeks. 47% of autografts fused.
Interbody fusion	goat (3) 1. rhBMP-2/ACS 2. Autograft	0.17 (0.43)	Radiography, histology and biomechanical testing: Radiographic scores higher for autograft than for rhBMP-2. Difference was not statistically significant.

IX.2.7.6 Preclinical Evaluations Conclusions

No evidence of any neurological abnormalities was found in studies performed to evaluate the safety of rhBMP-2/ACS in the vicinity of the spinal cord and nerve roots. In addition, there was no evidence, based on blood and cerebrospinal fluid analyses, of any clinical abnormalities in the animals tested. There was also no radiographic or histologic evidence of mineralization within the thecal sac.

In studies comparing fusion in the presence of rhBMP-2/ACS vs. autograft bone, the histological fusion rate was greater for the rhBMP-2/ACS treated animals than for the animals receiving autograft bone. In a study comparing rhBMP-2/ACS to ACS alone, all animals treated with rhBMP-2/ACS had solid interbody fusions while neither of the animals receiving ACS alone were fused. The animals receiving the higher dose of rhBMP-2 had qualitatively denser bone compared to those in the lower dose group. These studies indicate that rhBMP-2/ACS was able to induce interbody spinal fusion in a variety of interbody constructs.

rhBMP-2/ACS demonstrated effectiveness in promoting posterolateral spinal fusion using both open and minimally invasive surgical techniques.

In the studies in which the rhBMP-2/ACS or autogenous bone was placed inside a titanium alloy spinal fusion cage, the fusion rate of the rhBMP-2/ACS group was higher than that of the autograft control. When anterior plates were used instead of spinal fusion cages, the radiographic fusion scores of the rhBMP-2/ACS group were higher, but the difference was not statistically significant. These studies demonstrated the ability of

rhBMP-2/ACS to stimulate interbody fusion of the cervical spine in a manner at least equivalent to, if not better than autograft.

X. SUMMARY OF CLINICAL STUDIES

X.1 PILOT STUDY

Prior to initiating enrollment in a large-scale clinical trial, the sponsor conducted a small prospective, multi-center, non-blinded, randomized pilot study (14 subjects, 11 investigational and 3 control, at 4 sites). This study utilized the same study design, control and investigational devices, enrollment criteria, clinical and radiographic evaluation parameters and evaluation time points as the subsequent, large-scale study (these parameters are described in detail in Section X.2 below). The sponsor enrolled 14 subjects in this study - 7 open anterior surgical approach investigational subjects, 4 laparoscopic surgical approach investigational subjects and 3 open anterior surgical approach control subjects. At 48 months, data for 9 subjects (6 investigational and 3 control) were available for analysis.

X.1.1 Clinical and radiographic effectiveness parameters

Pain and function were assessed with the Oswestry Low Back Pain Disability Questionnaire and neurological status was assessed from an evaluation of muscle strength, reflexes, sensation and straight leg raises (SLR). The fusion endpoint was assessed using lateral flexion/extension films to evaluate the presence of motion and reconstructed sagittal CT scans to evaluate the presence of bridging trabecular bone. Plain radiographs were used to assess disc height and implant subsidence.

X.1.2 Clinical and radiographic effectiveness evaluation

At 24 months post-op, 91% (10/11) investigational and 100% (2/2) control subjects were determined to be successes in terms of their pain and function scores. By 48 months post-op, 1/2 control subjects with data had maintained their improvement from baseline pre-op levels. 4/7 investigational subjects maintained an improved score and 1 had a worse score. In addition, all but one subject (6 investigational, 2 control) reported less pain compared to their pre-baseline op scores with 5 of these subjects reporting no post-op pain.

At 24 month post-op evaluation, all investigational and control subjects were considered neurological successes in terms of motor, sensory and SLR scores. Only 9/11 (81.8%) investigational subjects were considered as successes for reflexes compared to 100% of the control subjects. At 48 months, 6 investigational subjects reported their overall postoperative neurological condition to be no worse than preoperatively.

All 11 investigational group subjects were found to be fused at 6, 12, and 24 months post-op. Only 2 of 3 control group subjects were determined to be fused at 6 months. At 18 months, the control subject not fused at 6 months underwent a second surgical intervention for implantation of supplemental fixation. At 48 months all 8 patients with radiographic data were fused.

X.1.3 Safety and immune response evaluation

For the investigational group, 8 of 11 subjects (72.7%) reported a total of 21 adverse events compared to 3 of 3 subjects (100%) for the control group. Three patients, 1 investigational and 2 control, were diagnosed as having pseudarthrosis. Other transient adverse events included urinary retention (1 control subject), numbness (1 investigational subject), facet joint pain (1 investigational subject), wound dehiscence (1 investigational subject) and back pain (2 investigational subjects). There were no episodes of retrograde ejaculation or anatomic and/or technical difficulties reported for the investigational laparoscopic approach group. No adverse events were reported at the 48 month visit.

Antibodies to rhBMP-2 and bovine and human Type I collagen were assessed. Antibodies were evaluated at three timepoints – pre-op and 6 weeks and 3 months post-op. None of the 14 subjects exhibited antibodies to rhBMP-2. Two subjects exhibited positive antibody responses to bovine Type I collagen at 6 weeks. Two of these remained positive at 3 months. No subjects were positive for antibodies to human Type I collagen. All subjects with positive antibody responses were determined to have radiographic fusion at their last examination.

X.2 PIVOTAL STUDY

X.2.1 Study Background

Clinical data to support the safety and effectiveness of the InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device were collected as part of a prospective, multi-center study that consisted of randomized and non-randomized arms. The randomized arm contained two groups, one investigational and one control. The control group was implanted with the LT-CAGE™ Lumbar Tapered Fusion Device filled with iliac crest autograft bone, while the investigational group was implanted with the InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device. In both cases, the surgical approach was an open anterior approach. The non-randomized arm contained only an investigational group, where subjects were implanted with the InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device through a laparoscopic anterior approach. The control group from the randomized arm was used as the control for the non-randomized arm.

Neither the investigators nor the subjects were blinded to the treatment. Subject blinding was not possible due to the second surgical site resulting from the need to collect the iliac crest grafts. The potential for investigator bias in the clinical outcome parameters was reduced by having the subjects rate their outcome using objective self-assessments. The radiographic outcome parameters were preformed by independent radiologists who were blinded to treatment. These were the only radiographic evaluations used for determining radiographic success.

X.2.2 Inclusion criteria

The indication studied was degenerative disc disease (DDD) accompanied by back pain with or without leg pain at a single level between L_4 and S_1 confirmed by history and

radiographic studies. DDD was determined to be present if one or more of the following were noted:

- instability (defined as angulation ≥ 5° and/or translation ≥ 4mm on flexion/extension radiographs);
- osteophyte formation;
- decreased disc height;
- ligamentous thickening;
- disc degeneration/herniation; or
- facet joint degeneration.

The following additional inclusion criteria had to be present:

- a pre-op Oswestry Low Back Pain Disability Questionnaire score of 35 or more;
- if spondylolisthesis were present, it could be no greater than grade I;
- be non-responsive to non-operative treatment for at least 6 months;
- skeletally mature; and
- not pregnant or nursing and agrees to the use of contraception for at least 16 weeks post-implantation.

X.2.3 Exclusion criteria

Subjects were excluded if they had any of the following:

- previous anterior spinal fusion at the involved level;
- posterior spinal instrumentation at the involved level or a previous interbody fusion procedure;
- any conditions that require postoperative medications that would be expected to interfere with fusion, *e.g.*, steroids, or has received drugs that interfere with bone metabolism within 2 weeks of surgery;
- osteoporosis, osteopenia, or osteomalacia;
- active malignancy;
- active local or systemic infection;
- gross obesity, defined as >40% IBW;
- fever >101°F;
- mentally incompetent;
- Waddell Signs of Inorganic Behavior ≥3;
- alcohol or drug abuse;
- tobacco user;
- an autoimmune disease;
- titanium allergy;
- previous exposure to injectable collagen implants;
- hypersensitivity to protein pharmaceuticals or collagen;
- previous exposure to rhBMP-2;
- allergy to bovine products or history of anaphylaxis;
- endocrine or metabolic disorder that affects osteogenesis; or
- received another investigational therapy within 28 days prior to implantation.

X.2.4 Post-operative care

The recommended post-operative care included the use of external immobilization for 6 weeks; abdominal strengthening exercises starting 30 days post-op; avoidance of repetitive bending, lifting, stooping, twisting and athletic activities until fusion had occurred; avoidance of prolonged NSAID use and prohibition of the use of electrical bone growth stimulators within 24 months post-op.

X.2.5 Clinical and radiographic effectiveness parameters

Patients were evaluated preoperatively (within 6 months of surgery), intraoperatively, and postoperatively at 6 weeks, 3, 6, 12 and 24 months and biennially thereafter until the last subject enrolled in the study had been seen for their 24 month evaluation. Complications and adverse events, device-related or not, were evaluated over the course of the clinical trial. At each evaluation timepoint, the primary and secondary clinical and radiographic outcome parameters were evaluated. Success was determined from data collected during the initial 24 months of follow-up. Antibodies to rhBMP-2 and bovine Type I collagen were assessed preoperatively and at 3 months post-operatively. Antibodies to human Type I collagen were assessed if the antibody response to bovine Type I collagen was positive.

Primary and secondary clinical and radiographic effectiveness outcome parameters were evaluated for all treated subjects at all follow-up evaluation timepoints identified above. The primary clinical parameters assessed were of pain, function and neurological status. The secondary clinical outcome parameters assessed were general health status, back and leg pain, donor site pain (control subjects only), patient satisfaction and patient global perceived effect of the treatment. The primary radiographic outcome parameter consisted of evaluations of fusion, while the secondary radiographic assessment was disc height.

Fusion was evaluated at 6, 12 and 24 months post-op using plain radiographs (AP, lateral and flexion/extension films) and high resolution thin-slice CT scans (1mm slices with 1mm index on axial sagittal and coronal reconstructions). Fusion was defined as the presence of bridging bone connecting the inferior and superior vertebral bodies; a lack of motion on flexion/extension ($\leq$ 3mm of translation and $< 5°$ of angulation); and no evidence of radiolucencies over more that 50% of either implant. Fusion success was defined as the presence of all of these parameters plus the lack of a second surgical intervention resulting from a non-union. All assessments were made from the plain films except for the assessment of bridging bone, which was made using the CT scans only if bridging bone could not be visualized on the plain film.

Pain and function were measured using the Oswestry Low Back Pain Disability Questionnaire. Success was defined as a 15 point improvement in the Oswestry score from the pre-op baseline score.

Neurological status consisted of measurements of four parameters - motor, sensory, reflexes, and SLR. Neurological status success was defined as maintenance or improvement of the pre-op baseline score for each parameter. Overall neurological status

success required that each individual parameter be a success for that subject to be counted as a success.

X.2.6 Patient demographics and accountability

The study was limited to 16 open surgical approach sites with 135 subjects per group (270 total open approach subjects) and 15 laparoscopic approach sites with 135 total subjects. This was subsequently modified to allow termination of enrollment of the open approach sites when 300 total subjects or 135 per group were reached. No changes were made to the laparoscopic approach site limits.

A total of 143 open approach investigational and 136 control patients were enrolled in the randomized arm of the study and received the device. A total of 134 subjects were enrolled in the non-randomized arm of the study and received the device. For the majority of the demographic parameters, there were no differences in pre-op demographics across the three populations.

Subject demographics			
	Investigational Open Surgical Approach	**Control Open Surgical Approach**	**Investigational Laparoscopic Surgical Approach**
n	143	136	134
men/women	78/65	68/68	57/77
mean age (range)	43.3 (22.4 – 78.4)	42.3 (19.0 – 70.6)	42.4 (19.2 – 69.5)
weight (lbs)	179.1	181.1	169.8
worker's comp (%)	47 (32.9)	47 (34.6)	42 (31.3)
tobacco user (%)	47 (32.9)	49 (36.0)	40 (29.9)
previous back surgery (%)	54 (37.8)	55 (40.4)	33 (24.6)

For the majority of the evaluation timepoints for all investigational groups, the follow-up rate was greater than 90%. For a small number of subjects, complete 24 month data for all effectiveness variables were not available, however. In order to "complete" the 24 month dataset for the subjects with missing data, 24 month values were predicted from the existing 12 month data using Bayesian statistical methods.

An analysis was performed to assess the ability to pool data across sites and to compare data across the study arms. These analyses evaluated the primary clinical outcome variables, as well as overall success and found no differences that would prevent pooling of the data across the sites within a given group of subjects.

Surgical results and hospitalization

Surgical and hospitalization information			
	Investigational Open Surgical Approach	**Control Open Surgical Approach**	**Investigational Laparoscopic Surgical Approach**
mean operative time (hrs)	1.6	2.0	1.9
mean EBL (ml)	109.8	153.1	146.1
hospitalization (days)	3.1	3.3	1.2
spinal level treated			
L_{45} (%)	37 (25.9)	32 (23.5)	21 (15.7)
L_5S_1 (%)	106 (74.1)	103 (75.7)	113 (84.3)
L_{56} (%)	0 (0)	1 (0.7)	0 (0)

While not statistically significant, the operative time and mean blood loss for the investigational open surgical approach subjects were lower than for the other two groups and the investigational laparoscopic surgical approach subjects required fewer hospitalization days.

X.2.7 Clinical and radiographic effectiveness evaluation

Individual subject success was defined as success in each of the primary clinical and radiographic outcome parameters. Success for these parameters included:

1. the presence of radiographic fusion;
2. an improvement of at least 15 points from the baseline Oswestry score;
3. maintenance or improvement in neurological status;
4. the presence of no serious adverse event classified as implant-associated or implant/surgical procedure-associated; and
5. no additional surgical procedure classified as "Failure."

Study success was expressed as the number of individual subjects categorized as a success divided by the total number of subjects evaluated. The table below describes the success rates for the individual primary outcome parameters and overall success. All success rates were based on the data from the 24 month follow-up evaluation and posterior probabilities of success were calculated using Bayesian statistical methods.

Posterior Probabilities of Success at 24 Months			
Primary outcome variable	**Investigational Open Surgical Approach**	**Control Open Surgical Approach**	**Investigational Laparoscopic Surgical Approach**
	Posterior Mean (95% HPD Credible Interval)	Posterior Mean (95% HPD Credible Interval)	Posterior Mean (95% HPD Credible Interval)
Fusion	92.8% (88.5%, 96.9%)	88.1% (82.6%, 99.3%)	93.0% (87.9%, 97.5%)
Oswestry	71.0% (63.4%, 78.7%)	70.9% (63.1%, 79.1%)	83.0% (75.6%, 90.5%)
Neurologic	81.0% (74.5%, 87.9%)	81.7% (74.9%, 88.7%)	89.0% (83.1%,94.8%)
Overall success	57.1% (49.2%, 65.7%)	56.7% (48.3%, 65.0%)	68.0% (59.3%, 76.5%)

The probability (also called the posterior probability) that the 24 month overall success rate for the investigational groups was equivalent to the 24 month success rate for the

control group was 99.4% for the open surgical approach investigational group and almost 100% for the laparoscopic surgical approach investigational group.

For a future patient receiving the InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device via the open anterior surgical approach, the chance (the predictive probability) of overall success at 24 months would be 57.1% for the open surgical approach. Given the results of the trial, there is a 95% probability that the chance of success ranges from 49.2% to 65.7% For a future patient receiving the InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device via the anterior laparoscopic surgical approach, the chance of overall success at 24 months would be 68.0%. Given the results of the trial, there is a 95% probability that the chance of success ranges from 59.3% to 76.5%. For a future patient receiving the control treatment, the chance of overall success at 24 months would be 56.7%. Given the results of the trial, there is a 95% probability that the chance of success ranges from 48.3% to 65.0%.

X.2.8 Safety and immune response evaluation

The assessment of safety consisted of an evaluation of the reported adverse events, as well as an evaluation of antibodies to rhBMP-2, bovine Type I collagen and human Type I collagen. The complete list of complications, adverse events and subsequent interventions is described in section VIII above. The presence of antibodies were assessed at the pre-op and 3 month post-op visits using ELISA. If there was a positive response to bovine Type I collagen, the serum was also tested for antibodies to human Type I collagen. The screening ELISA cutpoint for positive antibody responses was set to 5 times the standard deviation of sera from normal human donors. Subjects were considered to have an elevated immune response if the preoperative test was negative (titer < 50) and postoperative test was positive (titer ≥ 50) or if the preoperative test was positive and the postoperative test was positive with a three-fold higher titer than the preoperative test.

There were 3 subjects who had positive antibody responses to rhBMP-2 – 1 subject in each of the study groups. The rates of positive antibody response to rhBMP-2 were 0.7% in the open surgical approach investigational group and 0.8% in the laparoscopic surgical approach investigational and open surgical approach control groups. While there is a theoretical possibility that antibodies to rhBMP-2 could neutralize endogenous BMP-2, thereby interfering with subsequent bone healing, this was not observed during the course of the study.

Sixty-six subjects were considered to have an authentic elevated antibody response to bovine Type I collagen - 18 open surgical approach investigational subjects, 32 laparoscopic surgical approach investigational subjects and 16 control subjects. No subjects had positive responses to human Type I collagen.

An evaluation was performed on the impact of a positive antibody response on overall success and fusion success. There was very little difference in overall and individual success when antibody status was taken into consideration.

During the course of the study, 6 pregnancies were reported – one in the control group and five in the investigational groups. Two of the four pregnancies that occurred in the laparoscopic approach group resulted in first trimester miscarriages. The other three pregnancies in the investigational groups resulted in live births with no reported complications. None of the pregnant subjects had antibody responses to rhBMP-2 or Type I collagen (bovine or human), that were detectable to the limits of the sensitivity of the assay.

Two cases of cancer were diagnosed during the course of the pivotal study – one in an investigational group and one in the control group. An investigational subject was found to have pancreatic cancer while a control subject was found to have breast cancer. No additional information is available on these subjects, *e.g.*, BMP-2 receptor expression.

XI. CONCLUSIONS DRAWN FROM THE STUDIES

All of the data provided in the previous sections describing the preclinical and clinical studies provide reasonable assurance of the safety and effectiveness of the InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device when used by well-trained surgeons via anterior open or laparoscopic surgical approaches for spinal fusion procedures in skeletally mature patients with DDD at one level from L_4-S_1. DDD is defined as discogenic back pain with degeneration of the disc confirmed by patient history, function deficit and/or neurological deficit and radiographic studies. These DDD patients may also have up to Grade I spondylolisthesis at the involved level.

The study demonstrated that treatment of DDD with the InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device was as effective as the control treatment (the LT-CAGE™ Lumbar Tapered Fusion Device filled with iliac crest autograft). The results for the primary effectiveness outcome parameters for both investigational groups were equivalent to the control group. The InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device was able to achieve comparable clinical performance while avoiding the necessity of an iliac crest graft site and its associated pain.

XII. PANEL RECOMMENDATION

The PMA was reviewed at the Orthopedic and Rehabilitation Devices Advisory Panel meeting held on January 10, 2002. The Panel unanimously recommended to the FDA that the application for the InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device was approvable on the following conditions:

- the labeling should clearly identify:
    - the approved anterior open and laparoscopic surgical approaches;
    - the specific tapered spinal fusion cage to be used; and
    - the use of the device at a single involved lumbar spinal level; and
- the sponsor should perform the following nonclinical post approval studies:

- an evaluation of BMP receptor expression determination and *in vitro* proliferation evaluation in primary tumor cell isolates;

- an evaluation of the ability of rhBMP-2 antibodies to cause reproductive problems in all stages of fetal development from implantation to birth; and

- an evaluation of the response of mice to equal, multiple does of rhBMP-2 over a 1 year period.

XIII. CDRH DECISION

FDA concurred with the Panel's recommendations regarding additional studies involving determining primary tumor cell BMP receptor status and primary tumor cell *in vitro* responsiveness to rhBMP-2. In addition, FDA agreed with the Panel's recommendation for experiments to evaluate the potential for maternal antibodies to rhBMP-2 to interfere with embryonic development. The agency did not agree with the recommendation to evaluate toxic effects due to multiple doses of rhBMP-2 in mice over a one-year period for the following reasons:

- The sponsor had already conducted a one-year rat study and a 6-month dog study and found no device-related toxicities;

- The device is not intended to be implanted multiple times in prospective patients. The one-year rat study conducted by the sponsor evaluated the product under conditions analogous to that for which the product was intended to be used.

- The sponsor has conducted many animal studies to evaluate device performance. Repeat administration of rhBMP-2 in dogs and rats was evaluated to 28 days post-exposure. FDA believes that the rodent model suggested by the Panel would not provide additional safety information regarding the product that has not already been obtained from other studies.

As a result, the sponsor was required to perform only the first two studies recommended by the advisory panel. Specifically, the sponsor was required to perform:

- studies to assess the effects of rhBMP-2 on tumor promotion. These investigations included *in vitro* studies with primary tumor cell isolates. Observations from these studies could indicate a necessity to modify the device's labeling.

- studies to investigate the potential for an immune response to rhBMP-2 to interfere in embryonic development in rabbits. Observations from these studies could indicate a necessity to create a pregnancy monitoring database or modify the device's labeling.

In order to gather long-term safety and effectiveness data, the sponsor must conduct a post approval study to obtain six-year follow-up data from a statistically-justified number

of patients. Because of the unknown long-term device performance, particularly the resulting bony fusion characteristics, the post-approval study will include analysis of any retrieved implants.

Furthermore, the sponsor also agreed to develop the following assays in order to better define the immunological response to rhBMP-2 :

- development and validation of a new ELISA for rhBMP-2 that is capable of detecting all antibody isotypes; and

- development and validation of an assay which is capable of detecting neutralizing antibodies to rhBMP-2.

Finally, the sponsor was required to submit reports on three additional assays, *i.e.*, silver stained SDS-PAGE, Edmans test and glycoform analysis, to be added to the release specifications for the rhBMP-2 device component.

FDA worked with the sponsor to finalize product labeling and the requirements of the post approval studies. The sponsor's manufacturing facilities were inspected and were found to be in compliance with the Quality Systems Regulation (21 CFR 820).

FDA issued an approval order on July 2, 2002.

The InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device was granted expedited review status on January 12, 2001, because FDA believed that the possibility of reducing or removing the morbidity associated with the harvested autograft bone donor site through the use of a growth factor-soaked collagen sponge within the fusion cage offered an improvement to patient care.

XIV. APPROVAL SPECIFICATIONS

Directions for Use: See product labeling

Hazard to Health from Use of the Device: See Indications, Contraindications, Warnings, and Precautions, and Adverse Reactions in the labeling.

Post Approval Requirements and Restrictions: See the Approval Order.